A Low Renal Threshold for Glucose in Diabetic Patients with a Mutation in the Hepatocyte Nuclear Factor-1α (HNF-1α) Gene

R. Menzel*[1], P.J. Kaisaki[2], I. Rjasanowski[1], P. Heinke[3], W. Kerner[1], S. Menzel[2]

[1]*Klinikum Karlsburg, Karlsburg, Germany*
[2]*The Wellcome Trust Centre for Human Genetics, Oxford, UK*
[3]*Institute for Diabetes-Gerhardt Katsch, Karlsburg, Germany*

One form of maturity-onset diabetes of the young, Type 3 (MODY3), results from mutations in the gene coding for hepatocyte nuclear factor-1α (HNF-1α), a transcription factor first described in the liver. MODY3 is characterized by a defective glucose-stimulated insulin secretion. Earlier observations of glycosuria with normal blood glucose levels in some MODY families suggest an additional renal manifestation of the respective genetic defect.

We measured the renal threshold for glucose in five diabetic carriers of a missense mutation (Arg 272 His) in HNF-1α and, for comparison, in eight Type 1 diabetic patients, applying a non-invasive protocol of frequent parallel blood and urine sampling during a slow shift in blood glucose levels.

We found that the mean renal threshold for glucose was lowered in the HNF-1α diabetic patients compared to those with Type 1 diabetes (6.5 ± 0.9 mmol l^{-1} vs 10.7 ± 0.5 mmol l^{-1}; $p < 0.01$). This lowered glucose threshold might be an indication of an extra-pancreatic effect of HNF-1α gene mutations in humans. Defects in HNF-1α may lead to an altered tubular glucose reabsorption, possibly due to decreased expression of the renal glucose transporter proteins involved in reabsorption of glucose from the urine.

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Introduction

Maturity onset diabetes of the young (MODY)[1,2] is a monogenic form of diabetes mellitus. So far five subtypes, caused by mutations in different genes, have been identified.[3–7] Mutations in the homeodomain transcription factor hepatocyte nuclear factor-1α (HNF-1α) cause the subtype 3 (MODY3).[3] Prediabetic carriers of HNF-1α mutations show an impairment of glucose-stimulated insulin secretion[8] which leads to the development of overt diabetes usually before or during adolescence. Because this secretory defect is evident in prediabetic carriers, the primary pathophysiological lesion is thought to be in the insulin-secreting β-cells of the pancreas. Although HNF-1α is present in several tissues including liver and kidney,[9] there is thus far no evidence for the manifestation of HNF-1α defects in tissues other than the β-cells in humans. Mice lacking an active copy of the gene (HNF-1α 'knock out'), however, display a syndrome that includes severe hepatic dysfunction, phenylketonuria, and massive glycosuria.[10]

An excessive glycosuria was first reported by Tattersall[1,11] in diabetic patients from two MODY families, suggesting that a low renal threshold for glucose may be a feature of MODY in some families. At that time, subtypes of MODY characterized by a defined genetic defect were not yet distinguished.

The aim of the present study was to measure the renal threshold for glucose in patients from a MODY3 family with a history of conspicuous glycosuria in whom a missense mutation (Arg 272 His) in exon 4 of the gene is the cause of the diabetes.[12]

Patients and Methods

Patients

In members of the MODY3 family G16 (Figure 1), the presence of the Arg 272 His mutation was detected by direct sequencing of PCR products as described previously[12] or by digestion of the PCR product with the restriction endonuclease *Hha I*. The renal threshold for glucose was measured in the five diabetic members of

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* Correspondence to: Dr Ruth Menzel, Klinikum Karlsburg, Diabetes-zentrum, D-17495 Karlsburg, Germany. E-mail: ruth.menzel@greifswald.netsurf.de

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this family (Figure 1; Table 1) and in eight Type 1 diabetic patients (insulin dependent from diabetes onset, positive islet-cell and GAD antibodies, or fasting C-peptide lower than 0.08 nmol l^{-1}). Groups did not differ in age, BMI, albumin excretion rate (AER, <20 μg min^{-1} in all patients), or in the frequency of retinopathy. MODY patients had lower HbA_{1c} values, longer duration of the disease and received smaller daily insulin dosages ($p < 0.05$). All participants in the study gave informed consent. None of the females was pregnant.

For all six diabetic members of the G16 family, inpatient documents covering most of their diabetes history, including 24-h sampling of urine with corresponding blood glucose profiles (nine values per day), were analysed retrospectively, going as far back as 1947 (deceased ancestor F.F.). For the three mutation-positive children who are as yet non-diabetic, parallel blood and urine glucose values were measured; to date glucose tolerance tests have not been performed.

Determination of the Renal Threshold for Glucose

The method used for this determination was a modification of the method of Mohnike *et al.*[13]

Diabetes was controlled as usual up to the day before the test. The procedure was started at 6.00 am with the measurement of fasting blood glucose and glucose concentration in night urine, followed by the parallel assessment of glucose concentration in spontaneously voided urine and in blood every 30 min. If the urine was glucose-free in the third sample, a slow blood glucose increase was achieved by interrupting the insulin treatment and/or by giving 30 g of wholemeal bread until glucose appeared in urine (two HNF-1α and six Type 1 diabetic patients). If the third urine sample contained glucose, a slow blood glucose decrease was accomplished by either fasting and application of small doses of regular insulin, or by fasting and gentle physical activity until the urine became glucose-free (three HNF-1α and two Type 1 diabetic patients. The renal threshold for glucose was calculated as the mean of the two blood glucose values before the urine changed from glucose positive to negative or vice versa. The procedure lasted from 3 to 6 h, with between 7 and 14 parallel measurements of glucose in urine and blood taken (subjects drank 0.75–1.50 l mineral water or herbal tea).

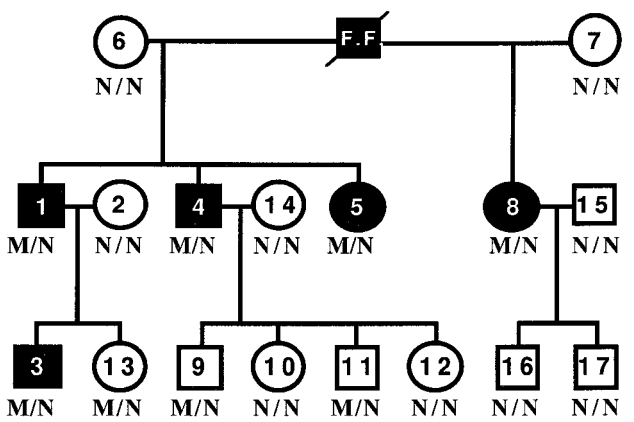

Figure 1. MODY 3 family G16. Diabetic and non-diabetic subjects are indicated by filled and open symbols, respectively. Clinical data for the diabetic subjects are presented in Table 1. Symbols: squares – males; circles – females. The HNF-1α genotype of each subject is indicated: N, normal allele; M, Arg 272 His mutation. Individuals 13 (9 years old), 9 (5 years old), and 11 (10 years old) are non-diabetic carriers of the mutation which reflects the age-dependent penetrance of HNF-1α mutations

We are convinced that the method used to obtain glucose threshold estimates in this outpatient study is sufficiently precise, as was also shown by Mohnike *et al.*[13] and by Mohnike and Worm.[14] We wished to avoid procedures involving glucose clamping or urinary catheterization out of consideration for our patients who would not have submitted to these procedures. Furthermore, stress as well as glucose infusions are known to alter tubular sodium reabsorption[15] and thus may confound glucose threshold determinations.

Biochemical Measurements

Capillary blood samples were taken for the immediate measurement of glucose with One Touch II (dry reagent technique based on glucose oxidase method – Lifescan/USA). The accuracy of each device was checked by a parallel measurement of at least three blood samples with a HemoCue photometer (Angelholm, Sweden). Glucose concentration in urine was measured in parallel with two visually evaluated strips (glucose oxidase method): Diabur 5000 (Boehringer Mannheim GmbH) and Diastix (Bayer Diagnostics, Munich). In four complete renal threshold tests, the glycosuria was additionally measured by reflectometry (clinitek 200, Bayer, Germany). AER was assessed in three overnight urine samples by nephelometry (Cobas Mira analyser, Roche, Switzerland). HbA_{1c} was determined by HPLC (Diamat, Bio Rad, Munich). Data are reported as mean ± SEM and were compared with the two-tailed Student's *t*-test.

Results

Renal Threshold for Glucose

The mean renal threshold for glucose of the patients with HNF-1α diabetes was lower than of those with Type 1 diabetes (Figure 2; 6.5 ± 0.9 mmol l^{-1}, individual values 5.1, 5.7, 5.7, 6.1 and 10.0 vs 10.7 ± 0.5 mmol l^{-1}, range 8.5–13.0; $p < 0.01$, Figure 2). The value of patient 8 (10.0 mmol l^{-1}) was confirmed in a second test. It may be relevant that she obtained near-normal glucose concentrations by exceptional care and insulin treatment from shortly after diagnosis, whereas her half-brother (patient 4), who exhibited the lowest renal

Table 1. Clinical characteristics of the HNF-1α diabetic subjects in family G16

Pedigree number (see Figure 1)	Sex	Age (years)	Diagnosis		Presentation	Therapy	Insulin therapy		HbA_{1c} % (normal range)	Complications
			At age	In year		*Current therapy*	Start after diagnosis in years	Current dosage (Units kg^{-1})		
F.F.	M	65 1971†	19	1925	S	2 weeks Synthalin *Insulin*	Temporary 18 Permanent 24	0.70		Blindness, amputation, renal insufficiency
1	M	35	6	1966	F	3 years Glibenclamide *Metformin*			6.4 (5.8–6.3)	
3	M	13	7	1990	S	*Insulin*	6	0.25	6.3 (5.8–6.3)	
4	M	34	4	1966	F	*Glibenclamide*			7.1 (3.4–6.0)	
5	F	39	6	1965	F	6 months Glibenclamide *Insulin*	9	0.30	5.3 (4.3–5.8)	Background retinopathy since 1980
8	F	42	12	1969	S	*Insulin*	Immediate	0.26	5.8 (4.4–6.4)	Background diabetic retinopathy since 1992

S, diabetic symptoms led to the diagnosis of the disease; F, family screening first revealed glycosuria and IGT, later overt diabetes developed.
†deceased.

threshold for glucose but refused insulin treatment, had the poorest diabetes control in the family (Table 1).

History of Diabetes and Glycosuria in the G16 Family

In the four HNF-1α diabetic patients with the low renal threshold for glucose, a conspicuously high glycosuria was evident in spite of relatively low blood glucose concentrations (<7 mmol l^{-1}) since the discovery of diabetes by family screening (Table 1).

In the ancestor F.F. a lowered renal threshold for glucose may also be assumed during the first decades of his diabetes, because a considerable glycosuria was observed frequently despite apparent physical well-being. Blood glucose testing, however, was rudimentary at that time. From 1947, in parallel with the development of chronic diabetic complications, evidence for a slow increase of the renal threshold for glucose up to about 9 mmol l^{-1} in 1963 can be derived from his medical records.

In contrast to the other patients in the family, there has been no indication of a low renal threshold for glucose in patient 8. The renal threshold can be inferred from the time insulin treatment started, i.e. 10 days after the sudden unexpected appearance of the disease with typical symptoms in 1969. In the week before the beginning of insulin treatment, the daily glycosuria varied between 30 and 50 g, but measurements of blood glucose (8.5 to 12.5 mmol l^{-1}) were too sporadic to infer the

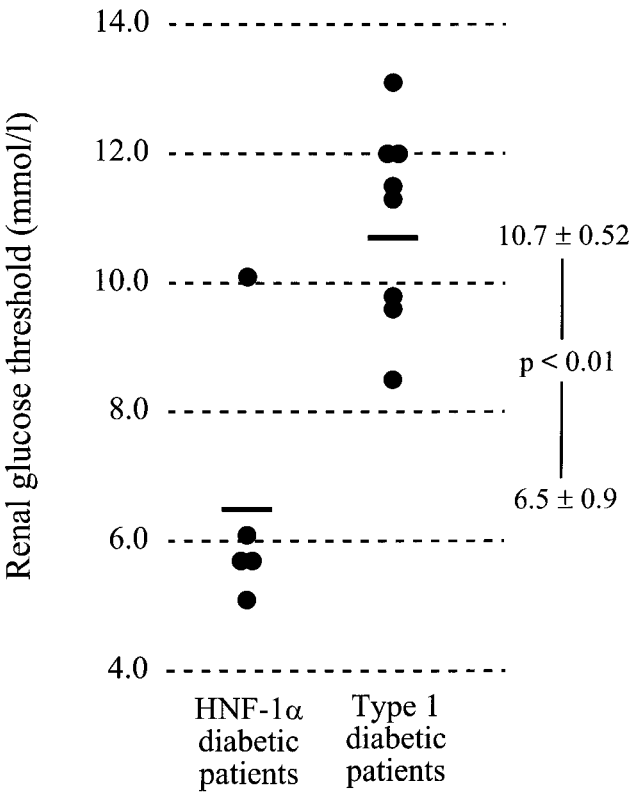

Figure 2. Renal threshold for glucose for HNF-1α diabetic members of family G16 (n = 5) and a group of Type 1 diabetic patients (n = 8)

renal threshold for glucose. So far, any indication of a renal disease has been absent.

Non-diabetic Children in the G16 Family

Here, the mean fasting and postprandial blood glucose values measured on four days were not different between the four mutation-negative (4.6 ± 0.2 and 5.5 ± 0.3 mmol l^{-1}, respectively) and the three mutation-positive (4.2 ± 0.1 and 5.9 ± 0.3 mmol l^{-1}) subjects. The corresponding urine samples were free of glucose, with the exception of a 9-year-old mutation-positive girl (subject 13) who once showed a single blood glucose value of 7.7 mmol l^{-1} together with traces of glucose in urine after she had had an opulent breakfast with sugar.

Discussion

We measured the renal threshold for glucose in five subjects with HNF-1α diabetes. The threshold values were significantly lower in these patients than those measured in eight Type 1 diabetic patients (6.5 ± 0.9 mmol l^{-1} vs 10.7 ± 0.5 mmol l^{-1}; $p < 0.01$). Our estimates for the Type 1 diabetic patients are in agreement with Mohnike *et al.*,[13] who found an average threshold estimate of 11.0 ± 0.2 mmol l^{-1} for 116 unselected insulin-treated diabetic patients (non-diabetic subjects 9.9 ± 0.2 mmol l^{-1}, $n = 5$).

Our results confirm those of Tattersall,[1] who observed that diabetic members of some MODY families showed glycosuria when normoglycaemic and inferred that the renal threshold for glucose was abnormally low in these families. Köbberling later coined the term 'Tattersall syndrome' for these and similar cases.[16] We believe that this alteration in renal function may be a feature of MODY3. To our knowledge, this kidney abnormality is the first indication of an extra-pancreatic effect of HNF-1α mutations in MODY3 patients.

A lowered renal threshold has been connected primarily to disturbances in tubular reabsorption, as in chronic pyelonephritis, toxin-mediated tubular damage or renal glycosuria.[17,18] Glucose reabsorption in the proximal convoluted tubule is achieved through the action of the active glucose – Na^+ cotransporters SGLT2 and SGLT1, and the facilitative glucose transporters GLUT2 and GLUT1. Defects in SGLT1 lead to intestinal monosaccharide malabsorption and intermittent glycosuria.[15] A disruption of the GLUT2 gene causes severe glycosuria and a defect in glucose sensing in insulin-producing β-cells.[19,20] Glucose transporter genes undergo changes in their renal expression levels in diabetic animal models. Secondary overexpression of GLUT2 in renal proximal tubules, as seen in the Zucker diabetic fatty rat,[21] may reflect adaptive mechanisms in response to a higher glomerular glucose load. We speculate that a decrease in the activity of HNF-1α might alter the expression of these genes, including diminishing their capacity for adaptation. So far it is not known whether HNF-1α is directly or indirectly involved in the regulation of glucose transporter genes. Further study of the HNF-1α knockout mice[10] and other animal models may help to uncover the mechanism of glycosuria in MODY3 patients.

The cause of the difference in renal glucose threshold seen between patients carrying the same HNF-1α mutation is so far unknown. Patient 8, the one with a normal glucose threshold, is conspicuous in three aspects in comparison to her half-siblings: her exceptional diabetes care might hint that a rigorous glucose control with application of insulin possibly influences the renal expression of the defect. On the other hand, she shares comparatively little of the genetic background with the rest of the family. This could imply that other genes are possibly modifying the impact of the HNF-1α mutation. Finally it should perhaps be considered that she may already have had diabetes long before it was diagnosed, which may have had chronic consequences for the kidney, although at present there are no signs of nephropathy.

So far it is unknown whether the occurrence of a lowered renal threshold for glucose in combination with diabetes is a feature of all patients with HNF-1α mutations and whether it is restricted to this subtype of diabetes. Overall, the molecular defects that can lead to conditions with excessive glucose excretion are not well understood. In renal glycosuria, a condition with excretion of glucose in urine with normal blood glucose and normal glucose tolerance, evidence for the existence of a single linked genetic locus is inconclusive, so that a variety of genetic alterations should be considered as possible causes.[15,18] In some patients diagnosed with renal glycosuria, especially in those which are later found to have developed diabetes mellitus,[22] HNF-1α mutations might be the underlying molecular defect.

The discovery that several broadly acting and widely distributed transcription factors, including HNF-1α, can be the cause of certain forms of diabetes is already beginning to alter our understanding of diabetes and its pathogenesis. In this context, the fact that the mutation in one of these genes might produce a phenotype in two different tissues is a further interesting aspect. Furthermore, we believe that our observation may be also significant for medical practice. An early detection of the onset of impaired glucose tolerance and an immediate and efficient therapy may help to prevent the severe chronic complications seen in MODY3 patients.[23–25] Because the conspicuous glycosuria seems to appear in the early stages of MODY, its detection should be one component of the necessary monitoring in young members of MODY families. It might be preferred as a simpler method in some cases, especially for small children.

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